PATENT ABSTRACTS OF JAPAN

(11)Publication number : 11-018793

(43)Date of publication of application : 26.01.1999

---

(51)Int.Cl. C12P 19/14

---

(21)Application number : 09-178057 (71)Applicant : UNITIKA LTD

(22)Date of filing : 03.07.1997 (72)Inventor : MORIMOTO AKIYOSHI
DONPOU MUNEHIKO
YOSHIKAWA GENICHI

---

(54) PRODUCTION OF MANNOBIOSE

(57)Abstract:
PROBLEM TO BE SOLVED: To efficiently obtain the subject saccharide useful as a raw material, etc., for foods, feed and medicines by reacting mannan, glucomannan, galactomannan or a natural product containing these materials with a mannan decomposing enzyme such as Aspergillus niger.
SOLUTION: Mannan, glucomannan, galactomannan or a natural product containing these materials (e.g. copra meal) is reacted with a mannan- decomposing enzyme derived from Aspergillus niger (e.g. galactomannanase) in an aqueous suspension at 50°C for 18 hr under stirring to carry out decomposition reaction of mannan, etc., and the reaction mixture is allowed to stand and the supernatant is filtered to afford a clear solution containing mannobiose. The solution is defatted with ethyl ether and passed through a porous type strongly basic anion exchange resin, a strongly acidic cation exchange resin and a weakly basic anion exchange resin in this order and the resultant solution is concentrated by an evaporator and crystallized by adding ethanol thereto to efficiently provide the objective mannobiose.

*CLAIMS

[Claim(s)]

[Claim 1]A manufacturing method of MANNO biose making a mannan dialytic ferment of Aspergillus-niger origin act on a natural product containing mannan, glucomannan, galactomannan, or these.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the manufacturing method of MANNO biose useful as foodstuffs, feed, or a drugs raw material.

[0002]

[Description of the Prior Art]D-mannose is the disaccharide which carried out the dyad glycosidic linkage, and MANNO biose carries out partial hydrolysis of the mannan contained in legumes, such as coconut coconut albumen, ivory coconut albumen, a needle-leaf tree, guar beans, and carob, konnyaku, etc. with acid or an enzyme, and is obtained by carrying out refining isolation from hydrolyzing liquid. Conventionally, the mannan dialytic ferment which the microorganism belonging to an Aspergillus, Bacillus, Streptomyces, and a Penicillium produces as an enzyme which cuts this glycosidic linkage is known. [tropical area agriculture (Japanese Journal of Tropical Agriculture) -- 29 (3), 167-172 (1985), and JP,8-2591948,B] .However, since the beta-mannanase activity of these enzymes was low, it was necessary to carry out a longtime reaction, therefore there was a problem that a manufacturing cost became high. Though the longtime reaction was carried out, it was not fully decomposed, but oligosaccharide generated, and there was a problem that the yield of MANNO biose was low.

[0003]It is reported to JP,63-209595,A that beta-mannanase of Penicillium origin separates MANNO biose from mannan, and hardly separates oligosaccharide, such as MANNO triose, further.

[0004]

[Problem(s) to be Solved by the Invention]However, even if it used beta-mannanase of

this Penicillium origin, there was a problem that the yield of MANNO biose was not enough. When using the MANNO biose manufactured with such enzymatic process for the use of foodstuffs, feed, etc., the microorganism which produces an enzyme will pose a problem also about the safety of the manufactured MANNO biose, if the safety as an object for food manufacturing is not established. An object of this invention is to provide the method of manufacturing MANNO biose with sufficient yield with enzymatic process from mannan and a mannan inclusion.

[0005]

[Means for Solving the Problem]In order to solve such a technical problem, wholeheartedly, as a result of examination, an enzyme of Aspergillus-niger origin acted on mannan etc., and this invention persons separated MANNO biose specifically, found out that MANNO biose can be obtained with sufficient yield, and reached this invention. That is, this invention makes a gist a manufacturing method of MANNO biose making a mannan dialytic ferment of Aspergillus-niger origin act on a natural product containing mannan, glucomannan, galactomannan, or these.

[0006]

[Embodiment of the Invention]Hereafter, this invention is explained in detail. As mannan and glucomannan which are used for this invention, and galactomannan (henceforth [these are named generically and] mannan), copra mannan, locust bean gum, guar gum, konjak mannan, etc. are mentioned. As a natural product containing these mannan, legumes, such as coconut coconut albumen, ivory coconut albumen, a needle-leaf tree, guar beans, and carob, a konnyaku potato, etc. are mentioned. In this invention, although these natural products may be used as they are and mannan may be extracted and used from these natural products, operation of extracting mannan is complicated, and since it becomes still higher [a manufacturing cost], using as it is is preferred.

[0007]As an enzyme used for this invention, it is of Aspergillus-niger origin, and if there is activity which acts on mannan and separates MANNO biose, it will not be limited in particular. The liquefying amylase by which Aspergillus niger is used for starch processing, It is used for many years as a production bacillus of enzymes, such as hemicellulase used for the improvement in extractability of the pectinase used for fruit-juice clear, the protease used for food processing, and coffee, and is the bacillus in which safety was established.

[0008]Such an enzyme can be obtained by cultivating Aspergillus niger. It is not limited especially if it is a method by which a mannan dialytic ferment is produced, and what is necessary is just to use the usual culturing methods, such as solid culture and liquid

culture, as the method of culture. What is necessary is just to cultivate on condition of usual also as a culture condition. In this invention, what kind of fraction containing mannan decomposition activity may be used, and refining or the thing which carried out partial purification can also be used for the fraction which contains mannan decomposition activity if needed with a conventional method.

[0009]A commercial enzyme agent may be used and SUMICHIMU ACH, SUMICHIMU AC (all are Shin Nippon Kagaku Industries), etc. are mentioned as such an enzyme agent. These enzyme agents are marketed as a food-grade enzyme agent, there is an operating experience as a food-grade way, and safety is established. SUMICHIMU ACH is one sort of the hemicellulase which Aspergillus niger produces, and has the following physicochemical properties.

(1) Although it has an operation and substrate specificity **-galactosidase activity, and **-galactosidase activity, beta-mannosidase activity is low or hardly has activity. It acts on mannan and galactomannan and MANNO biose is generated.

(2) Optimum pH and operation pH optimum pH are 4.5, and act practical in between [pH 3.0 to 6.0].

(3) The optimal temperature of the range reaction of operation optimal temperature acts practical between 30 ** - 70 ** at 60 **.

(4) Make into 1/100 unit (unit) enzyme activity which reduces by half the viscosity of 1 ml of roast bean gum solution (pH 4.5) of the measurement relative viscosity 6 [about] of potency in 1 minute at 40 **. The enzyme activity of this commercial enzyme is 50,000 units /g as galacto mannanase activity.

[0010]It also has hemicellulase activity, although SUMICHIMU AC is cellulase of Aspergillus-niger origin. A physicochemical property is shown for this enzyme below.

(1) It has an operation, substrate specificity cellulose, and hemicellulose activity. It acts on mannan and MANNO biose is generated.

(2) Optimum pH and operation pH optimum pH are 4.5, and act practical in between [pH 3.0 to 5.5].

(3) The optimal temperature of the range reaction of operation optimal temperature acts practical between 30 ** - 70 ** at 60 **.

(4) Use measurement carboxymethylcellulose sodium (pH 5.0) of potency as a substrate, and make into one unit (unit) 40 ** and enzyme activity which separates the reducing sugar of 1micromol in 1 minute. The enzyme activity of this commercial enzyme is 2,000 units /g as cellulase activity.

[0011]If it is the conditions used for the usual enzyme reaction as conditions which make the above-mentioned enzyme act on the natural product containing mannan or

mannan, there will be no problem in particular, but it is desirable to react on the optimal operation conditions of the enzyme to be used. Although it is desirable to react as a temperature of a reaction under the conditions on which an enzyme is not deactivated, in order to prevent putrefaction of reaction mixture, reacting at the temperature which a microorganism cannot increase easily is desirable, and 40-80 ** 50-75 ** of 20-90 ** is still more preferably good preferably. moreover -- it cannot be overemphasized that reacting under the optimum operation conditions of an enzyme is desirable as pH of a reaction -- pH 2-9 -- desirable -- pH 2.5-8 -- pH 3-6 is still more preferably good. Although it depends for reaction time on the quantity of the enzyme to be used, it is preferred on work that 3 to 48 hours usually sets up.

[0012]Thus, the obtained MANNO biose can be refined using the various chromatography which used ion-exchange resin and activated carbon further. It can be made to crystallize by making it powdered by spray drying, or adding ethanol.

[0013]

[Example]Next, an example explains this invention concretely.

After the example 1 copra meal 200g (10% of fat, 7.2% of moisture) was suspended in the water of 2L, 1g of SUMICHIMU ACH (the galacto mannanase by Shin Nippon Kagaku Industries, potency 50,000 unit /g) was added, and it was made to react under 18-hour churning at 50 **. After ending reaction, it settled and the clear solution 1.9L containing MANNO biose was obtained by filtering a supernatant fluid. High-speed liquid column chromatography analyzed the sugar in this solution. Using Shimazu LC-6A, column temperature was derivatized by 60 ** and rate-of-flow 0.4 ml/min, the column for analysis derivatized the mobile phase in the boric acid buffer solution (pH 8.7) of 0.5M, and a post column, and the fluorescence detector detected it. The content of MANNO biose was calculated from the fixed-quantity value of the reference standard. As a result, 42 g of MANNO biose was being accumulated into 1.9L.

[0014]porous mold strongly-basic-anion-exchange-resin PAafter degreasing this solution with ethyl ether 308 [subsequently,] (the Mitsubishi Chemical make.) a Cl - type, 100 ml of bed volume, and strongly-acidic-cation-exchange-resin SK1B (the Mitsubishi Chemical make.) It dipped in an H+ type, 100 ml of bed volume, and weakly-basic-anion-exchange-resin WA30 (the Mitsubishi Chemical make, an OH - type, 100 ml of bed volume) in this order, and the solutions containing MANNO biose were collected. The collected solution was condensed by the evaporator until it became Brix 70, and the honeydew 112g containing MANNO biose was obtained. When 50 ml of ethanol was added to this concentrate and it was crystallized, 39 g of MANNO biose was obtained.

[0015]After the example 2 copra meal 200g (10% of fat, 7.2% of moisture) was suspended in the water of 2L, 2g of SUMICHIMU AC (cellulase by Shin Nippon Kagaku Industries, potency 2,000 unit /g) was added, and it was made to react under 24-hour churning at 60 **. After ending reaction, it settled and the clear solution 1.9L containing MANNO biose was obtained by filtering a supernatant fluid. As a result of conducting analysis of the sugar in this solution with high-speed liquid column chromatography like Example 1, 38 g of MANNO biose was being accumulated into 1.9L.

[0016]porous mold strongly-basic-anion-exchange-resin PAafter degreasing this solution with ethyl ether 308 [subsequently,] (the Mitsubishi Chemical make.) a Cl - type, 100 ml of bed volume, and strongly-acidic-cation-exchange-resin SK1B (the Mitsubishi Chemical make.) It dipped in an H+ type, 100 ml of bed volume, and weakly-basic-anion-exchange-resin WA30 (the Mitsubishi Chemical make, an OH - type, 100 ml of bed volume) in this order, and the solutions containing MANNO biose were collected. After condensing the collected solution by an evaporator until it became Brix 70, ethanol was added so that it might become last concentration 85 capacity %, a little crystal D-MANNO bioses were added, and it was neglected at 4 **. By filtering this solution, 20 g of crystal MANNO biose was obtained. The melting point of the obtained MANNO biose was 191-192 **.

[0017]After the example 3 locust bean gum 200g was suspended in the water of 2L, 1.6g of SUMICHIMU ACH (the galacto mannanase by Shin Nippon Kagaku Industries, potency 50,000 unit /g) was added, and it was made to react under 18-hour churning at 55 **. The clear solution 1.9L containing MANNO biose was obtained by filtering after ending reaction. As a result of conducting analysis of the sugar in this solution with high-speed liquid column chromatography like Example 1, 80 g of MANNO biose was being accumulated into 1.9L.

[0018]subsequently, this solution -- porous mold strongly-basic-anion-exchange-resin PA308 (the Mitsubishi Chemical make.) a Cl - type, 100 ml of bed volume, and strongly-acidic-cation-exchange-resin SK1B (the Mitsubishi Chemical make.) It dipped in an H+ type, 100 ml of bed volume, and weakly-basic-anion-exchange-resin WA30 (the Mitsubishi Chemical make, an OH - type, 100 ml of bed volume) in this order, and the solutions containing MANNO biose were collected. The white powder 126g containing MANNO biose was obtained by carrying out spray drying of the collected solution.

[0019]After 200 g of example 4 guar gum was suspended in the water of 2L, 1.6g of SUMICHIMU ACH (the galacto mannanase by Shin Nippon Kagaku Industries,

potency 50,000 unit /g) was added, and it was made to react under 18-hour churning at 55 **. The clear solution 1.9L containing MANNO biose was obtained by filtering after ending reaction. As a result of conducting analysis of the sugar in this solution with high-speed liquid column chromatography like Example 1, 60 g of MANNO biose was being accumulated into 1.9L.

[0020]subsequently, this solution -- porous mold strongly-basic-anion-exchange-resin PA308 (the Mitsubishi Chemical make.) a Cl - type, 100 ml of bed volume, and strongly-acidic-cation-exchange-resin SK1B (the Mitsubishi Chemical make.) It dipped in an H+ type, 100 ml of bed volume, and weakly-basic-anion-exchange-resin WA30 (the Mitsubishi Chemical make, an OH - type, 100 ml of bed volume) in this order, and the solutions containing MANNO biose were collected. The white powder 112g containing MANNO biose was obtained by carrying out spray drying of the collected solution.

[0021]

[Effect of the Invention]According to this invention, MANNO biose useful as foodstuffs, feed, and a drugs raw material can be manufactured with sufficient yield.

[Translation done.]